Establishing left-right (LR) asymmetry is required for correct position and function of internal organs. Although remarkable progress has been made in understanding the molecular basis of LR establishment, how this molecular information is translated into morphogenetic events is unknown. To elucidate the cellular mechanisms that control asymmetric morphogenesis, we have analyzed the initial LR asymmetric morphogenesis of the early heart rudiment, a process called C-looping, in chick and mouse embryos. Our detailed analysis with time-lapse microscopy revealed that this process is achieved by two independent, asymmetric morphogenetic events: rightward rotation of the rostral portion of the primitive heart tube and asymmetric growth of its caudal portion. Interestingly, we found that cell shape and cell arrangements undergo similar changes during asymmetric morphogenesis of both parts. This finding suggests that common molecular and cellular mechanisms function to generate different LR asymmetric morphological features in the rostral and caudal regions of the looping heart tube. Based on our histological and genetic analyses, we will discuss what molecules and cell behaviors are regulated by LR signals and how common molecular machinery could control differential morphogenesis in these two regions of the heart tube. Our study sheds light on the mechanisms that enable LR signals to control complex morphogenesis.

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Program/Abstract # 83
The KRAB Zinc Finger Protein ZFP568 is Required in the Extraembryonic Mesoderm for Yolk Sac and Placental Development

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In mammals, extraembryonic tissues are critical for sustaining embryonic life inside the uterus, providing nourishment and secreting factors to maintain pregnancy. *chato*, an ENU allele disrupting the mouse Kruppel-associated box (KRAB) zinc finger protein ZFP568, causes unique defects in the morphogenesis of extraembryonic tissues. The yolk sac of *chato* mutants contains bubble-like protrusions, accompanied by a detachment of the visceral endoderm from the underlying extraembryonic mesoderm, and defective extraembryonic mesoderm migration. Additionally, the placenta fails to develop properly in *chato* mutants. Most *chato* embryos have an expanded chorionic ectoderm that, in extreme cases, prevents the closure of the ectoplacental cavity. Also, development of the labyrinth layer of the placenta is disrupted in all *chato* mutants. Interestingly, we found that the severity of yolk sac defects correlated with the placental malformations, suggesting that all extraembryonic defects in *chato* mutants have a common developmental origin. To address the requirements of *Zfp568* in different extraembryonic lineages, we analyzed chimeric embryos generated by both tetraploid complementation assays and by the use of a reversible allele of *Zfp568* in combination with Cre lines. Our results indicate that ZFP568 is required in the extraembryonic mesoderm to regulate the development of the yolk sac and placenta. Results will be presented that support a previously undescribed role of the extraembryonic mesoderm in the morphogenesis of extraembryonic tissues.

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Program/Abstract # 84
Live-imaging analysis of apoptosis and caspase activation reveals that apoptosis is a facilitator of the neural tube closure

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Neural tube closure (NTC) is a fundamental process to construct the brain and the spinal cord in vertebrates. The neural plate develops bilateral neural folds, which in turn come into contact in the midline and fuse together to create the neural tube. Programmed cell death (PCD), especially apoptosis, is widely observed during the process. Several lines of evidence suggested that apoptosis is important for NTC. However, it is still unclear how apoptosis contributes to the completion of NTC. To examine the relationship between dying cells and morphogenetic movement such as neural plate bending, fusion of neural ridge, and epithelial remodeling after the fusion, direct visualization of dying cells during NTC could be effective. We have conducted a live-imaging analysis of apoptosis during NTC with the mouse embryo expressing SCAT3, a fluorescent indicator protein to monitor caspase-3 activation in living cells. In these embryos, caspase activation was observed in dying cells of the pre-fusion neural ridge, fusion point, and the midline. Thus, this system allowed us to observe the dynamics of NTC when caspase activation and apoptosis were inhibited. We found that the speed of closure tended to become slow under caspase-inhibited condition, suggesting that caspase activation and apoptosis contribute to the progression of NTC within a limited developmental time window.

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Program/Abstract # 85
Apoptosis is dispensable for the control of cell number during early brain morphogenesis

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Apoptosis is essential for normal brain morphogenesis. Mice lacking genes required for apoptosis (*caspase-3*, *caspase-9*, and *apaf-1*) exhibit severe brain malformations such as small brain ventricles and folded neuroepithelium. Because these morphological changes led to the idea that the supernumerary cells are generated in these mutants' brain, it has been thought that apoptosis is essential to control the brain cell number. However, quantitative changes in total cell number of these mutants' brain have not been investigated. So, we performed quantitative morphological analysis of *apaf-1* mutants' brain. We measured the area of neuroepithelium and the cell density in serial sections and counted the total cell numbers of dissociated brain tissue. To our surprise, there was no detectable increase in the cell number of *apaf-1* mutants' brain, even when the typical brain malformations were apparent. The influence of apoptosis inhibition on the brain cell number in *apaf-1* mutant embryos might be diminished by some other cell number-controlling mechanisms, such as alternative cell death programs or suppression of cell proliferation. In contrast, *apaf-1* mutant embryos failed to expand its brain ventricles, and this may be the cause of neuroepithelial deformations. Completion of neural tube closure is thought to be required for the drastic brain ventricles expansion. Indeed, *apaf-1* mutant embryos exhibited neural tube closure defects, suggesting that the primary